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Effect of inulin on the growth and metabolism of a probiotic strain of *Lactobacillus rhamnosus* in co-culture with *Streptococcus thermophilus*

Ricardo Pinheiro de Souza Oliveira [a,*], Patrizia Perego [b], Maricê Nogueira de Oliveira [a], Attilio Converti [b]

[a] *Biochemical and Pharmaceutical Technology Department, São Paulo University, São Paulo 05508-900, Brazil*
[b] *Department of Chemical and Process Engineering, Genoa University, Genoa University, Genoa I-16145, Italy*

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ABSTRACT

Metabolic studies are very important to improve quality of functional dairy products. For this purpose, the behaviors of pure cultures of *Streptococcus thermophilus* (St) and *Lactobacillus rhamnosus* (Lr) as well a co-culture of them (St–Lr) were investigated during skim milk fermentation, and the inulin effect as prebiotic was assessed. Lr was able to metabolize 6 g/100 g more galactose than St and St–Lr. Final lactic acid production by Lr was higher (9.8 g/L) compared to St (9.1 g/L) and St–Lr (9.1 g/L). Acetic acid concentration varied from 0.8 g/L (St–Lr) to 1.5 g/L (Lr) and that of ethanol from only 0.2 g/L (St–Lr) to 0.4 g/L (Lr), which suggests the occurrence in Lr of a NADH oxidase activity and citrate co-metabolization via pyruvate, both dissipating a part of the reducing power. Diacetyl and acetoin accumulated at the highest levels (18.4 and 0.8 mg/L, respectively) with St–Lr, which suggests possible synergistic interactions between these microorganisms as well as the Lr capability of co-metabolizing citrate in the presence of lactose. Inulin stimulated both biomass growth and levels of all end-products, as the likely result of fructose release from its partial hydrolysis and subsequent metabolization as an additional carbon and energy source.

1. Introduction

The various health benefits of probiotic bacteria mainly belonging to the *Lactobacillus* and *Bifidobacterium* genera have led to their increased incorporation in yoghurts and fermented milks (Ashraf & Shah, 2011), ice creams and pharmaceutical products (Mattila-Sandholm et al., 2002).

To enhance their therapeutic effects, dairy foods usually contain also prebiotics, i.e. non-digestible oligosaccharides that resist hydrolysis and absorption in the upper gastrointestinal tract and are metabolized selectively by at least one type of probiotic in the colon (Mattila-Sandholm et al., 2002). Among these, inulin was shown to exert a protective effect on lactic acid bacteria (LABs) by stimulating their survival and activity during storage of the final product (Donkor, Nilmini, Stolic, Vasiljevic, & Shah, 2007). It is a soluble and fermentable fructan that cannot be digested by α-amylase or other hydrolytic enzymes (Villegas & Costell, 2007) and is mainly applied to get low-fat products (Oliveira, Florence et al., 2009).

Although the metabolic response of homofermentative and heterofermentative LABs to environmental conditions is well documented (Axelsson, 1998), there is scarce information on the metabolism of probiotics in co-cultures. Among LABs, lactobacilli are classified as gram-positive, non-sporulating, catalase-negative, acid-tolerant, anaerobic fermentative bacteria with different sensitivity to oxygen and a reputed Generally Recognized as Safe (GRAS) status (Kleerebezem & Hugenholtz, 2003). Sugars are fermented by the homofermenters mainly to lactic acid, while the heterofermenters yield, in addition to lactic acid, a large variety of other fermentation products such as acetic acid, ethanol, CO_2 and formic acid (Gomes & Malcata, 1999; Kleerebezem & Hugenholtz, 2003), diacetyl, acetoin and 2,3-butanediol from citrate, bioactive peptides from the catabolism of amino acids, and exopolysaccharides from carbohydrates (Mayo et al., 2010). All these compounds contribute to the sensorial and nutritional properties of fermented products.

Lactobacillus rhamnosus is a facultative heterofermentative bacterium that ferments hexoses such as lactose and fructose to lactic acid, and also pentoses to a mixture of lactic and acetic acids (Hammes & Vogel, 1995). In addition, *L. rhamnosus*, as other LABs, co-metabolizes citrate to 4-carbon compounds, such as diacetyl, acetoin and 2,3-butanediol, which have flavoring properties and impart the typical aroma to many dairy products (Helland, Wicklund, & Narvhus, 2004). Thus, it may be a possible candidate for industrial production of these flavoring compounds (Jyoti, Suresh, & Venkatesh, 2004).

* Corresponding author. Tel.: +5511 30912478; fax: +5511 3815 638.
E-mail address: rpsolive@usp.br (R.P. de Souza Oliveira).

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Most of the "thermophilic" LABs preferentially metabolize the glucose moiety of lactose, after its transport and cleavage by β-galactosidase, while galactose is mainly excreted in the medium, resulting in a galactose-negative phenotype (Axelsson, 1998; Svensson, Lohmeier-Vogel, Waak, Svensson, & Rådström, 2007; de Vin, Rådström, Herman, & de Vuyst, 2005). Such behavior was ascribed either to a low galactokinase activity (Hickey, Hillier, & Jago, 1986) or to an energetically favorable reaction of lactose transport system (Hutkins & Ponne, 1991). Other LABs, among those used in this study, have greater ability to metabolize galactose, thereby resulting in a galactose-positive phenotype (Mayo et al., 2010; Tsai & Lin, 2006).

To get advance in this field, the associative behaviors of *Streptococcus thermophilus* with *L. rhamnosus* have been investigated on the basis of the following assumptions: a) hydrolysis of lactose, b) lactic acid formation from glucose and partially from galactose, c) release of unmetabolized galactose, d) diacetyl and acetoin formation, and e) biomass growth. Finally, the effect of inulin as prebiotic has been assessed by comparing the results of fermentations carried out either with or without it.

2. Materials and methods

2.1. Microorganisms

Two strains (Danisco, Sassenage, France) were used in this study, specifically *S. thermophilus* TA040 (St) and *L. rhamnosus* LBA (Lr).

2.2. Milk preparation

Milk was prepared by adding 13 g of skim powder milk (Castroni, Reggio Emilia, Italy) in 100 g of distilled water without or with 40 mg of inulin/g (trade name: Beneo TM) (Orafti Active Food Ingredients, Oreye, Belgium). The above solid content of milk corresponds to the average value reported by Restle, Pacheco, and Moletta (2003) for whole cow milk, while the selected inulin concentration was in the range (3–6 g/100 g) admitted by the Brazilian legislation on yoghurt (ANVISA, 2002). Both milks were thermally treated at 90 °C for 5 min in water bath, model Y14 (Grant, Cambridge, United Kingdom). Heated milks were transferred to 1.0-L sterile flasks, cooled in ice bath, distributed into 250-mL sterile Schott flasks inside a laminar flow hood, and stored at 4 °C for 24 h before use.

2.3. Inoculum preparation

The *L. rhamnosus* pre-culture was prepared by dissolving 130 mg of freeze-dried culture in 50 mL of milk (10 g/100 g of total solids; autoclaved at 121 °C for 20 min). After blending and activation at 42 °C for 30 min, 1.0 mL of the pre-culture was inoculated in 500 mL-Erlemeyer flasks containing 250 mL of skim milk. The *S. thermophilus* pre-culture was prepared in the same way by adding 90 mg of its freeze-dried culture to 50 mL of milk. Counts of these pre-cultures ranged from 6.1 to 6.5 logCFU/mL.

2.4. Fermentations

After inoculation, the flask content was transferred to a 3.0 L-fermenter, model Z61103CT04 (Applikon, Schiedam, The Netherlands) with 2.0 L-working volume and provided with an electronic device, model ADI1030 (Applikon). The dissolved oxygen concentration was measured by a sterilized galvanic electrode, InPro6000 Series (Mettler-Toledo, Novate Milanese, Italy). Batch fermentations were carried out at 42 °C independently, in triplicate, without any agitation, and stopped when the pH reached 4.5, according to the common practice in yoghurt manufacture.

2.5. Counts of viable bacteria

Cell counts were made by plating in triplicate after fermentation, as previously described (Oliveira, Perego, Converti, & Oliveira, 2009). Samples (1.0 mL) were added to 9.0 mL of 0.1 g/100 g sterile peptonated water; then, appropriate dilutions were made. Subsequently, *S. thermophilus* was plated into M17 Agar (Oxoid, Basingstoke, UK) and then submitted to aerobic incubation at 37 °C for 48 h (Dave & Shah, 1996). *L. rhamnosus* was counted in MRS Agar, with pH adjusted to 5.4 by addition of acetic acid, after jar anaerobic incubation at 37 °C for 72 h (Lankaputhra & Shah, 1996). Anaerobic conditions were ensured in an oxoid jar with the Anaerogen (Oxoid) system. Colony forming units (CFU) were enumerated in plates containing 30 to 300 colonies, and cell concentration was expressed as logCFU/mL of fermented milk.

2.6. Analytical methods

After dilution of samples and casein precipitation by acidification to pH 4.5 with HCl (Hipp, Groves, Custer, & McMeekin, 1950), biomass concentration was determined by optical density (OD) measurements at 640 nm using a UV–Vis spectrophotometer, model Lambda 25 (Perkin Elmer, Wellesley, MA), and a calibration curve of OD against dry weight. For dry weight determinations, cells were harvested by centrifugation in Eppendorf tubes, washed twice with distilled water and dried to constant weight at 70 °C.

A high-performance liquid chromatograph, model 1100 (Hewlett Packard, Palo Alto, CA), was used to analyze lactose, glucose, galactose, acetic acid, diacetyl, acetoin, ethanol and lactic acid. The system consisted of an HP-1050 Intelligent Auto Sampler, an HP-1047A Refractive Index Detector, an HP-1050 UV Detector and an HP-1050 pump. Separation was achieved using a Supelcogel H59304-U column (Sigma Aldrich, Bellefonte, PA) at 50 °C with 0.01 mol/L sulfuric acid as eluent at 0.4 mL/min flowrate. The column was calibrated for at least 3 h before use, utilizing the same solution under the same conditions as the separation.

3. Results and discussion

3.1. Acidification profiles

Fig. 1 shows the acidification profiles of milk (A) and milk supplemented with 40 mg of inulin/g (B) by pure cultures of *S. thermophilus* (St) and *L. rhamnosus* (Lr) and a co-culture of *S. thermophilus* with *L. rhamnosus* (St–Lr) at 42 °C until reaching pH 4.5. It should be noted that the time to complete the fermentation depended not only on inulin addition but also on possible interactions between these two microorganisms. In the presence of inulin, the time to complete the fermentations by the St–Lr co-culture and the pure cultures of St and Lr was 48.1, 13.9 and 8.7% shorter than without inulin, respectively (panel A). Such a marked effect demonstrates that inulin stimulated the metabolism of both microorganisms, thus confirming its prebiotic effect already reported for lactobacilli (Donkor et al., 2007; Makras, van Acker, & de Vuyst, 2005; Oliveira, Florence et al., 2009). The very long fermentation time of pure Lr culture (15.0 h) could have been due either to the need of this microorganism to co-metabolize citrate or to the inducible feature of its citrate transport system (Jyoti et al., 2004), while the quicker fermentation by the co-culture with respect to the single cultures could have been the result of synergistic effects between St and Lr.

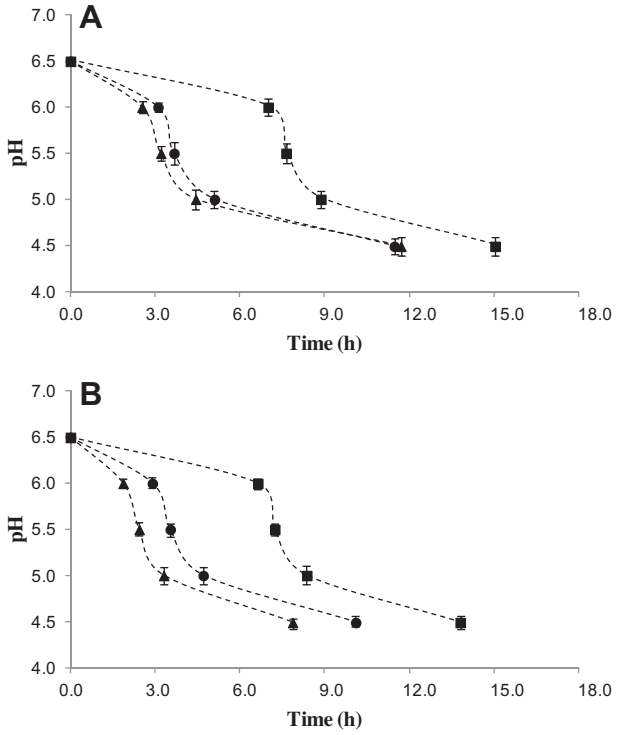

Fig. 1. Acidification profiles of skim milk fermentations by pure cultures of *S. thermophilus* (●) and *L. rhamnosus* (■) or a co-culture of *S. thermophilus* with *L. rhamnosus* (▲), in the absence (A) or in the presence (B) of 40 mg inulin/g at 42 °C until reaching pH 4.5.

3.2. Metabolites formation and growth

Figs. 2 and 3 show the fermentation behavior in skim milk of St, Lr, and St–Lr, without and with 40 mg of inulin/g, respectively.

The most evident characteristics of these fermentations are: (1) the higher growth of *S. thermophilus* with respect to *L. rhamnosus*, (2) the partial consumption of lactose, (3) the formation of lactic acid as the major metabolic product, and of acetic acid and ethanol as typical co-products of heterolactic fermentation, (4) the release of galactose, as the result of its slow metabolization, and (5) the accumulation of diacetyl and acetoin in the medium at very low levels.

Fig. 2 clearly shows that both mono-cultures as well as the co-culture fermented mainly the glucose moiety of lactose, while a relevant portion of galactose was excreted in the medium. However, the pure culture of Lr was shown to metabolize 6 g/100 g more galactose than that of St and the St–Lr co-culture. This behavior may be explained by the weak transcription from gal promoters or mutations in the Leloir genes by many strains of *S. thermophilus* (de Vin et al., 2005). Moreover, according to Tsai and Lin (2006), in *L. rhamnosus*, the galactose moiety of lactose could be metabolized also by two alternative pathways, specifically the Leloir and the tagatose 6-phosphate pathways. As a result, the final production of lactic acid by the Lr pure culture was little higher (9.8 g/L) than by both the St pure culture (9.2 g/L) and the St–Lr co-culture (9.2 g/L).

Besides lactic acid, other typical co-products of heterolactic fermentation were also produced, mainly acetic acid and ethanol. Fig. 2 shows that the level of acetic acid varied from 0.8 g/L (St–Lr) to 1.5 g/L (Lr) and that of ethanol from only 0.2 g/L (St–Lr) to 0.4 g/L (Lr). Since *S. thermophilus* is a homofermentative bacterium, its fast metabolism was mainly responsible for the production of lactic acid, while the formations of acetic acid and ethanol have to be

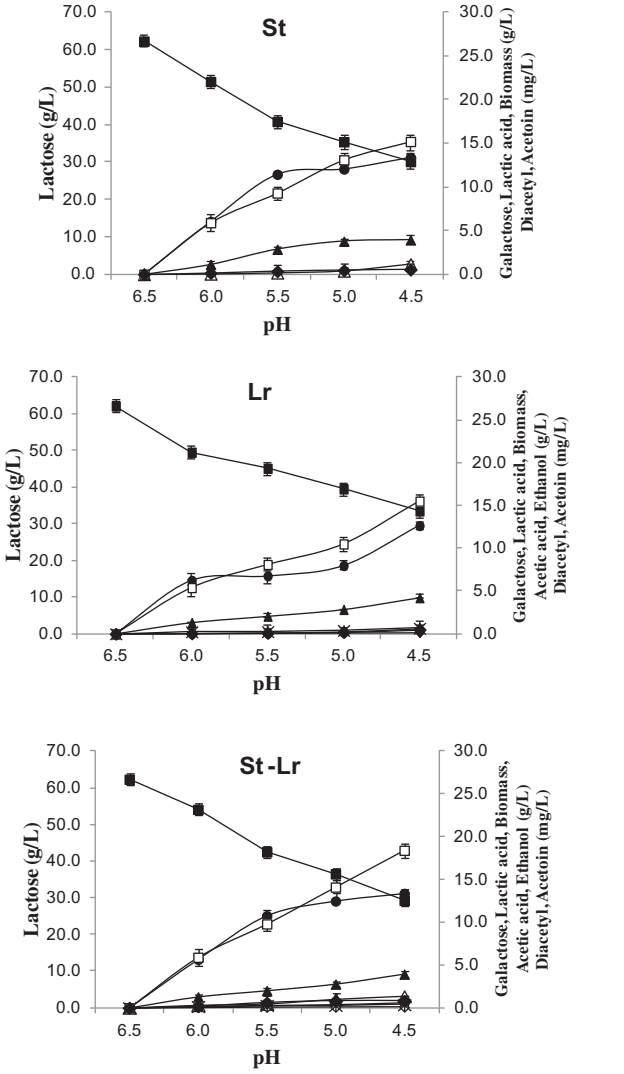

Fig. 2. Concentration versus pH profiles of skim milk fermentations by pure cultures of *S. thermophilus* (St) and *L. rhamnosus* (Lr) or a co-culture of *S. thermophilus* with *L. rhamnosus* (St–Lr) in the absence of inulin. Concentrations of lactose (■); galactose (●); lactic acid (▲); St biomass (△); Lr biomass (○); acetic acid (*); ethanol (◇); diacetyl (□); acetoin (◆).

ascribed to the heterofermentative feature of *L. rhamnosus*. It is well known that, in a typical heterofermentative pathway, glucose from lactose hydrolysis, and in some microorganisms even a portion of the remaining galactose moiety, are converted via phosphoketolase to glyceraldehydes 3-phosphate and acetyl-CoA, being the former converted to lactic acid and the latter reduced to ethanol by the NADH accumulated in the first part of the pathway (Axelsson, 1998). Under oxidative conditions, such an excess reducing power can be partially dissipated, and an appreciable amount of acetyl-P can be converted to acetic acid making the phosphoketolase pathway as efficient as the EMP one from the bioenergetic viewpoint (Arsköld et al., 2008; Zaunmüller, Eichert, Richter, & Unden, 2006). As the formation of acetic acid yields an additional equivalent of ATP (Axelsson, 1998), it is less energy-consuming; therefore, the presence in the medium of additional hydrogen acceptors is needed to sustain its abundant production as in the present work. As we will see in the following, the concentration of acetoin from diacetyl reduction was too low to justify this production; thus, two possible explanations of such a partial dissipation of NADH could be the co-metabolization of citrate via pyruvate, with additional

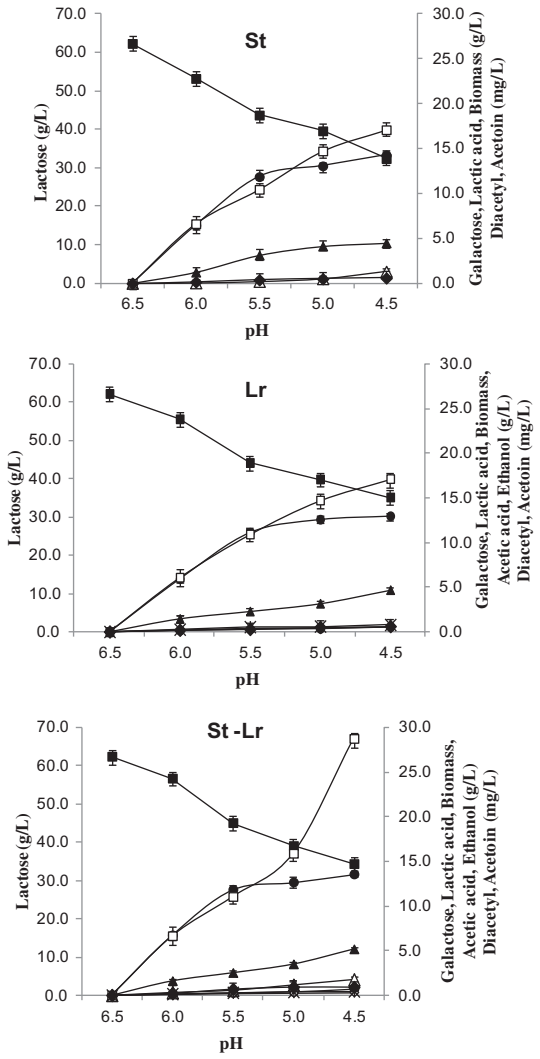

Fig. 3. Concentration versus pH profiles of skim milk fermentations by pure cultures of *S. thermophilus* (St) and *L. rhamnosus* (Lr) or a co-culture of *S. thermophilus* with *L. rhamnosus* (St–Lr) in the presence of inulin. Concentrations of lactose (■); galactose (●); lactic acid (▲); St biomass (△); Lr biomass (○); acetic acid (*); ethanol (◇); diacetyl (□); acetoin (◆).

formation of lactic acid (Axelsson, 1998), and the high NADH oxidase activity already detected and quantified in *L. rhamnosus* by Jyoti et al. (2004) by metabolic flux analysis.

In pure cultures, the productions of diacetyl and acetoin by *L. rhamnosus* (Lr) were 18 and 67% higher, respectively, when compared to those obtained with *S. thermophilus* (St). Ramos, Jordan, Cogan, and Santos (1994) demonstrated that in LABs the main route of diacetyl synthesis occurs via α-acetolactate, which is produced by the condensation of two pyruvate molecules catalyzed by the key enzyme α-acetolactate synthase. Once synthesized, α-acetolactate is unstable and is readily decarboxylated to acetoin by α-acetolactate decarboxylase, or by nonenzymatic oxidative decarboxylation to diacetyl, in the presence of oxygen. Besides that, acetoin can be synthesized from diacetyl by diacetyl reductase; so, the balance among the end-products of citrate fermentation will depend on the redox state of the cell.

As *S. thermophilus* cannot metabolize citrate (Chaves et al., 2002), the poor formation of these flavoring compounds in mono-culture suggested the occurrence of a shift from homolactic metabolism to a mixed-acid one under stress conditions such as carbon limitation, excess of slowly metabolizable sugars, and so on (Mayo et al., 2010). So, the productions of both diacetyl and acetoin by *S. thermophilus*, for which α-acetolactate synthase and decarboxylase activities are well documented (Monnet & Corrieu, 2007), can be ascribed in the present work to lactose metabolization.

On the other hand, it has been reported that, through citrate permease induction by citrate, several LAB species are able to metabolize citrate (Mayo et al., 2010) producing 4-carbon compounds such as diacetyl and acetoin. In addition, *L. rhamnosus* was shown to co-metabolize citrate at low or intermediate levels only in the presence of a fermentable sugar such as lactose (Jyoti, Suresh, & Venkatesh, 2003). Additional pyruvate is formed during citrate metabolism, so that most of it becomes available when required to oxidize the NADH released during sugar fermentation (Axelsson, 1998). Since citrate is present in significant amounts in milk of many animals, like cows and goats (1.5 g/L) (Linzell, Mepham, & Peakert, 1976), the presence of the above flavoring compounds in our fermented products was supposed to be the likely result of citrate fermentation.

The highest values of diacetyl (18.4 mg/L) and acetoin (0.8 mg/L) were obtained at the end of the St–Lr co-culture (Fig. 2), which suggests the occurrence of a synergism between St and Lr, leading to great advantages in the manufacture of dairy products because of their characteristic flavors. According to Oliveira, Perego, Oliveira, and Converti (2011), the increased presence of these flavoring end-products in co-cultures could be ascribed to substantial metabolic changes. These results taken together demonstrate that *L. rhamnosus* could be a possible candidate to industrially synthesize diacetyl and acetoin.

Another synergistic effect is evidenced in Fig. 2 by the higher increase in biomass concentration in the co-culture compared with pure cultures. St and Lr did in fact exhibit maximum final cell concentrations 15.5 and 44% lower than in St–Lr, respectively. One hypothesis to explain such an effect is that *S. thermophilus* produces small amounts of formic acid and CO_2 (Mayo et al., 2010) that can stimulate the growth of other LABs, while *L. rhamnosus* is able to release peptides by a serine protease of the subtilisin family (known as PrtR) that stimulate the growth of *S. thermophilus* (Siezen, 1999).

3.3. Effect of inulin as a prebiotic

As shown in Fig. 3, the presence of inulin enhanced, in general, the levels of all main metabolic end-products. In particular, at the end of fermentation, the concentration of lactic acid in the St pure culture, Lr pure culture and St–Lr co-culture was 1.2, 10.9 and 26.1% higher than without inulin (Fig. 2). By the same reasoning, the acetic acid concentration increased by 21.5% in the Lr pure culture and 33.5% in the St–Lr co-culture, and that of ethanol by no less than 300% in the Lr pure culture and 241% in the St–Lr co-culture.

These results point out a generalized stimulation of the overall metabolism of LABs induced by inulin, as the likely result of fructose release from its partial hydrolysis and subsequent metabolization as an additional carbon and energy source (Oliveira et al., 2011). Perrin, Warchol, Grill, and Schneider (2001) did in fact report that the paradigm for prebiotic action is that probiotics possess cell-associated glycosidases that hydrolyze oligosaccharides. In the case of fructooligosaccharides (FOS), like inulin, such an enzyme is a fructofuranosidase (Imamura, Hisamitsu, & Kobashi, 1994). Monosaccharides other than glucose can be fed into the phosphoketolase pathway, and *L. rhamnosus* was already shown to ferment fructose (Forouhandeh, Vahed, Hejazi, Nahaei, & Dibavar, 2010). Similarly, *Lactobacillus paracasei*, which belongs to the same group of facultatively heterofermentative bacteria as *L. rhamnosus* (Hammes & Vogel, 1995), produced significant amounts of lactic acid, acetic acid, formic acid, and ethanol when

long-chain inulin or oligofructose-enriched inulin was used as the sole energy source (Makras et al., 2005).

So, the increased levels of ethanol and acetic acid induced by inulin in both Lr mono-culture and St–Lr co-culture suggests that some fructose released from partial inulin hydrolysis was likely to be heterofermented by Lr. Moreover, the larger increase in ethanol level compared to that of acetic acid suggests that the microorganism could have utilized the acetyl-CoA hydrogenation to ethanol as a way to dissipate the excess NADH resulting from possible inhibition of NADH oxidase activity by inulin.

4. Conclusions

The present work dealt with the effect of inulin on the growth and metabolism of a probiotic strain of *L. rhamnosus* (Lr) in mono-culture or in co-culture with *S. thermophilus* (St).

These fermentations were mostly characterized by higher growth of St compared to Lr, a partial consumption of lactose, the formation of lactic acid as the major metabolic product, and of acetic acid and ethanol as typical co-products of heterolactic fermentation of sugars, the release of galactose as the result of its slow metabolization, and the accumulation of diacetyl and acetoin in the medium at very low levels.

In pure cultures, the productions of diacetyl and acetoin by Lr were 18 and 67% higher, respectively, when compared with St. In fact, whereas in the latter microorganism these flavoring compounds derive from lactose metabolism, in the former diacetyl and acetoin syntheses occur via α-acetolactate, and acetoin can also be synthesized from diacetyl by diacetyl reductase.

Final cell concentrations of St and Lr were remarkably lower in pure cultures (by 15.5% and 44%, respectively) compared with the co-culture, thus confirming the occurrence of a synergism between these two microorganisms. In addition, inulin remarkably stimulated the growth of all cultures.

The time to complete the fermentations was reduced not only by the inulin addition but also by interactions between St and Lr. Certainly, fructose from partial inulin hydrolysis was utilized as an additional carbon and energy source homofermentatively by St and heterofermentatively by Lr, and consequently the concentrations of all fermentation products were enhanced.

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References

Agência Nacional de Vigilância Sanitária - ANVISA. (2002). *Legislação. VisaLegis. Resolução RDC n.2, de 7 de janeiro de 2002. Aprova o regulamento técnico de substâncias bioativas e probióticos isolados com alegação de propriedades funcional e ou de saúde*. January 9th and July 17th, 2002. Brasilia: Diario Oficial da União.

Arsköld, E., Lohmeier - Vogel, E., Cao, R., Roos, S., Rådström, P., & Van-Niel, E. W. (2008). Phosphoketolase pathway dominates in *Lactobacillus reuteri* ATCC 55730 containing dual pathways for glycolysis. *Journal of Bacteriology, 190*, 206–212.

Ashraf, R., & Shah, N. P. (2011). Selective and differential enumerations of *Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium* spp. in yoghurt - a review. *International Journal of Food Microbiology, 149*, 194–208.

Axelsson, L. T. (1998). Lactic acid bacteria; classification and physiology. In S. Salminen, & A. von Wright (Eds.), *Lactic acid bacteria* (pp. 1–72). New York: Marcel Dekker.

Chaves, A. C., Fernández, M., Lerayer, A. L., Mierau, I., Kleerebezem, M., & Hugenholtz, J. (2002). Metabolic engineering of acetaldehyde production by *Streptococcus thermophilus*. *Applied and Environmental Microbiology, 68*, 5656–5662.

Dave, R. I., & Shah, N. P. (1996). Evaluation of media for selective enumeration of *Streptococcus thermophillus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus* and *bifidobacteria*. *Journal of Dairy Science, 79*, 1529–1536.

Donkor, O. N., Nilmini, S. L. I., Stolic, P., Vasiljevic, T., & Shah, N. P. (2007). Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. *International Dairy Journal, 17*, 657–665.

Forouhandeh, H., Vahed, S. Z., Hejazi, M. S., Nahaei, M. R., & Dibavar, M. A. (2010). Isolation and phenotypic characterization of *Lactobacillus* species from various dairy products. *Current Research in Bacteriology, 3*, 84–88.

Gomes, A. M. P., & Malcata, F. X. (1999). *Bifidobacterium* spp. and *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends in Food Science & Technology, 10*, 139–157.

Hammes, W. P., & Vogel, R. F. (1995). The genus Lactobacillus in the lactic acid bacteria. In B. J. B. Wood, & W. H. Holzapfel (Eds.), *The genera of lactic acid bacteria* (pp. 19–54). London, UK: Blackie Academic.

Helland, M. H., Wicklund, T., & Narvhus, J. A. (2004). Growth and metabolism of selected strains of probiotic bacteria in milk- and water-based cereal puddings. *International Dairy Journal, 14*, 957–965.

Hickey, R. W., Hillier, A. J., & Jago, G. R. (1986). Transport and metabolism of lactose, glucose and galactose in homofermentative lactobacilli. *Applied and Environmental Microbiology, 51*, 825–831.

Hipp, N. J., Groves, M. L., Custer, J. H., & McMeekin, T. L. (1950). Separation of γ-casein. *Journal of the American Chemical Society, 72*, 4928–4931.

Hutkins, R. W., & Ponne, C. (1991). Lactose uptake driven by galactose efflux in *Streptococcus thermophilus*: evidence for a galactose-lactose antiporter. *Applied and Environmental Microbiology, 57*, 941–944.

Imamura, L., Hisamitsu, K., & Kobashi, K. (1994). Purification and characterisation of β-fructofuranosidase from *Bifidobacterium infantis*. *Biological and Pharmaceutical Bulletin, 17*, 596–602.

Jyoti, B. D., Suresh, A. K., & Venkatesh, K. V. (2003). Diacetyl production and growth of *Lactobacillus rhamnosus* on multiple substrates. *World Journal of Microbiology and Biotechnology, 19*, 509–514.

Jyoti, B. D., Suresh, A. K., & Venkatesh, K. V. (2004). Effect of preculturing conditions on growth of *Lactobacillus rhamnosus* on medium containing glucose and citrate. *Microbiological Research, 159*, 35–42.

Kleerebezem, M., & Hugenholtz, J. (2003). Metabolic pathway engineering in lactic acid bacteria. *Current Opinions in Biotechnology, 14*, 232–237.

Lankaputhra, W. E. V., & Shah, N. P. (1996). A simple method for selective enumeration of *Lactobacillus acidophilus* in yoghurt supplemented with *L. acidophilus* and *Bifidobacterium* spp. *Milchwissenschaft, 51*, 446–451.

Linzell, J. L., Mepham, T. B., & Peakert, M. (1976). The secretion of citrate into milk. *The Journal of Physiology, 260*, 739–750.

Makras, L., van Acker, G., & de Vuyst, L. (2005). *Lactobacillus paracasei* subsp. *paracasei* 8700:2 degrades inulin-type fructans exhibiting different degrees of polymerization. *Applied and Environmental Microbiology, 71*, 6531–6537.

Mattila-Sandholm, T., Myllärinen, P., Crittenden, R., Mogensen, G., Fondén, R., & Saarela, M. (2002). Technological challenges for future probiotic foods. *International Dairy Journal, 12*, 173–182.

Mayo, B., Aleksandrzak-Piekarczyk, T., Fernández, M., Kowalczyk, M., Pablo Álvarez- Martín, P., & Bardowski, J. (2010). Updates in the metabolism of lactic acid bacteria. In F. Mozzi, R. R. Raya, & G. M. Vignolo (Eds.), *Biotechnology of lactic acid bacteria: Novel applications* (pp. 3–33). Iowa, USA: Wiley-Blackwell.

Monnet, C., & Corrieu, G. (2007). Selection and properties of α-acetolactate decarboxylase-deficient spontaneous mutants of *Streptococcus thermophilus*. *Food Microbiology, 24*, 601–606.

Oliveira, R. P. S., Florence, A. C. R., Silva, R. C., Perego, P., Converti, A., Gioielli, L. A., et al. (2009). Effect of different prebiotics on the fermentation kinetics, probiotic survival and fatty acid profiles in nonfat symbiotic fermented milk. *International Journal of Food Microbiology, 128*, 467–472.

Oliveira, R. P. S., Perego, P., Converti, A., & Oliveira, M. N. (2009). Effect of inulin on growth and acidification performance of different probiotic bacteria in co-cultures and mixed culture with *Streptococcus thermophilus*. *Journal of Food Engineering, 91*, 133–139.

Oliveira, R. P. S., Perego, P., Oliveira, M. N., & Converti, A. (2011). Effect of inulin as a prebiotic to improve growth and counts of a probiotic cocktail in fermented skim milk. *LWT-Food Science and Technology, 44*, 520–523.

Perrin, S., Warchol, M., Grill, J. P., & Schneider, F. (2001). Fermentations of fructo-oligosaccharides and their components by *Bifidobacterium infantis* ATCC 15697 on batch culture in semi-synthetic medium. *Journal of Applied Microbiology, 90*, 859–865.

Ramos, A., Jordan, K. N., Cogan, T. M., & Santos, H. (1994). C Nuclear magnetic resonance studies of citrate and glucose cometabolism by *Lactocuccus lactis*. *Applied Environmental Microbiology, 60*, 1739–1748.

Restle, J., Pacheco, P. S., & Moletta, J. L. (2003). Genetic group and postpartum nutritional level on the milk yield and composition of beef cows. *Revista Brasileira de Zootecnia, 32*, 585–597.

Siezen, R. J. (1999). Multi-domain, cell-envelope proteinases of lactic acid bacteria. *Antonie Van Leeuwenhoek - International Journal of General and Molecular Microbiology, 76*, 139–155.

Svensson, M., Lohmeier-Vogel, E., Waak, E., Svensson, U., & Rådström, P. (2007). Altered nucleotide sugar metabolism in *Streptococcus thermophilus* interferes

with nitrogen metabolism. *International Journal of Food Microbiology, 113*, 195–200.

Tsai, Y. K., & Lin, T. H. (2006). Sequence, organization, transcription and regulation of lactose and galactose operons in *Lactobacillus rhamnosus* TCELL-1. *Journal of Applied Microbiology, 100*, 446–459.

Villegas, B., & Costell, E. (2007). Flow behaviour of inulin–milk beverages. Influence of inulin average chain length and of milk fat content. *International Dairy Journal, 17*, 776–781.

de Vin, F., Rådström, P., Herman, L., & de Vuyst, L. (2005). Molecular and biochemical analysis of the galactose phenotype of dairy *Streptococcus thermophilus* strains reveals four different fermentation profiles. *Applied and Environmental Microbiology, 71*, 3659–3667.

Zaunmüller, T., Eichert, M., Richter, H., & Unden, G. (2006). Variations in the energy metabolism of biotechnologically relevant heterofermentative lactic acid bacteria during growth on sugars and organic acids. *Applied Microbiology and Biotechnology, 72*, 421–429.